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CASE REPORT

NON-INVASIVE SCREENING TEST PARADOX IN A CASE BORN WITH MIXED GONADAL DYSGENESIS (45,X/46,XY)

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ABSTRACT

Noninvasive prenatal testing (NIPT) is commonly used to screen for fetal trisomy 13, 18, and 21 and often for sex chromosomal aneuploidies (SCAs). Although the testing is also used for sex chromosomal aneuploidies, it is not as efficient as it is for common trisomies. In this particular study, we present a case for whom the NIPT diagnosis was originally 45,X and who was diagnosed with mixed gonadal dysgenesis 45,X /46,XY after birth. A 38-year-old [G3P3] pregnant woman underwent NIPT at 15 weeks' gestation and was found to be at probable risk for 45,X. Because cordocentesis is an invasive procedure, the pregnant woman did not want to undergo cordocentesis. Consequently, postnatal cytogenetic analysis was performed and the baby's karyotype was shown to be 45,X/46,X,+mar?. No numerical and/or structural anomalies were observed in the karyotypes of parents and siblings. Based on the microarray analysis of the analyzed sample, one copy of the X chromosome was detected in all cells and the presence of one copy of the Y chromosome was detected in a ~40% mosaic state: arr(X) x1,(Y)x1[0.4]. *SRY* gene duplication on Y chromosome was confirmed by fluorescence in situ hybridization (FISH) and microarray analysis. The patient's clinical examination showed ambiguous genitalia (clitoromegaly) and dysmorphic facial features. The baby underwent surgery for aortic coarctation. The results were consistent with a genetic diagnosis of 45,X /46,XY mixed gonadal dysgenesis. Genetic counselling was offered to the family. In conclusion, NIPT still has potential limitations in correctly identifying sex chromosomes and mosaicism that may mislead clinicians and families.

Keywords: Mixed gonadal dysgenesis, mosaicism, noninvasive prenetal testing, prenatal diagnosis, Turner syndrome

INTRODUCTION

Turner syndrome (TS) is a chromosomal disorder commonly observed in females and caused by structural or numerical abnormalities of the X chromosome. TS affects 1 in 2500 live births [1]. Patients diagnosed with Turner syndrome have a unique phenotype that includes a webbed neck, broad chest, and low posterior hairline. In addition, structural cardiac abnormalities, gonadal dysgenesis, hypertension and diabetes are some of the secondary sex characteristics observed in patients with Turner syndrome [2,3]. Nearly 40-60% of TS patients have a 45,X karyotype, on the other hand, 45,X/46, XX; 46,X,i(Xq); and other variants are observed in several TS patients [1]. The gene responsible from short stature is found in the pseudoautosomal region 1 (PAR1), which is located on the short arm of the X chromosome and it is defined as the short stature homeobox-containing (*SHOX*) gene [4].

Furthermore, mixed gonadal dysgenesis can be observed in females with Turner syndrome who have 45,X/46,XY mosaicism or sex-determining region Y (*SRY*) gene [5]. The presence of a whole Y chromosome or Y-derived material is observed in a range of 4% to 61% in TS patients with different karyotypes [6]. Mixed gonadal dysgenesis (MGD) comprises a heterogeneous group of different chromosomal, gonadal, and phenotypic abnormalities characterized by the presence of a testis on one side and a contralateral stripe or absent gonad. Therefore, the phenotype varies from normal male patients to patients with ambiguous external genitalia to female patients [7,8].

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Cytogenetic analysis is routinely performed for the genetic diagnosis of Turner syndrome. However, in recent years, molecular techniques such as polymerase chain reaction and single nucleotide polymorphism (SNP) genotyping have been used to detect sex chromosome abnormalities in a very short time with a low cost, using both blood and buccal samples. Especially with the recent improvements in technology, array comparative genomic hybridization (acGH) has been used to understand the genetic basis of Turner Syndrome [1].

Cell-free-non-invasive prenatal testing (cfNIPT) has been widely used as a screening for fetal trisomy 13, 18, and 21, and it is also used for the screening of sex chromosomal aneuploidies (SCAs) by analyzing cell-free fetal DNA (cffDNA) in maternal plasma [7,9]. In addition, improvements in molecular genomics enabled the use of NIPT to screen for copy number variants (CNVs) and various single gene disorders [10]. It has been shown that cfNIPT is highly sensitive and specific for trisomiy 21 (sensitivity: >99%), trisomy 13 (sensitivity: >98%), and trisomy 18 (sensitivity: >99%) [7]. However, the concurrence is lower for SCAs (from 90.5 to 100%). Moreover, the positive predictive value (PPV) for sex chromosomal anomalies is lower than for common trisomies, ranging from 9% to 40% [4,11].

The mosaic Turner Syndrome may be under-diagnosed due to several reasons, such as subtle phenotypic characteristics and technical problems [12]. This is mainly observed in patients who have low rates of mosaicism due to an increased number of euploid cells or may be described as an artifact that may affect the true genetic diagnosis of Turner Syndrome [1].

In the literature, it has been emphasized that low levels of fetus-driven cell-free DNA concentration in the blood is one of the limitations of NIPT while detecting sex chromosomal anomalies. More importantly, the performance of NIPT in detecting mosaicism has not been adequately studied. In this report, we present a case in which the NIPT diagnosis was originally 45,X and the patient was diagnosed with mixed gonadal dysgenesis 45,X /46,XY after birth**.**

CASE REPORT

A 38-year-old pregnant woman [G3P3] was admitted to our clinic. A non-invasive prenatal diagnosis test (NIPT) was performed at the 15th week of pregnancy. The results of the NIPT indicated a probable risk for 45,X. Due to the lockdowns at the beginning of the COVID-19 pandemic, cordocentesis was offered at the 22nd week of pregnancy. At that time, she presented to our clinic in the 26th week of pregnancy. The family was informed that cordocentesis is an invasive procedure and that there is a high risk of losing the baby during cordocentesis. For this reason, cordocentesis was not performed. After birth, karyotyping of the peripheral blood and the epithelial cells of the oral mucosa were performed and the karyotype of the baby was determined as 45,X/ 46,XY+mar? respectively. No numerical and/or structural anomalies were observed in the karyotypes of parents and siblings. Based on the microarray analysis of the analyzed sample, one copy of the X chromosome was detected in all cells and the presence of one copy of the Y chromosome was detected in a ~40% mosaic state: arr(X)x1,(Y)x1[0.4].

Lastly, *SRY* gene duplication on Y chromosome was detected by Fluorescent *in situ* Hybridization (FISH) and microarray analysis. Clinical examination of the patient showed ambiguous genitalia (clitoromegaly) and an abnormal facial shape. Also was shown that the newborn was phenotypically female, with the exception of neonatal

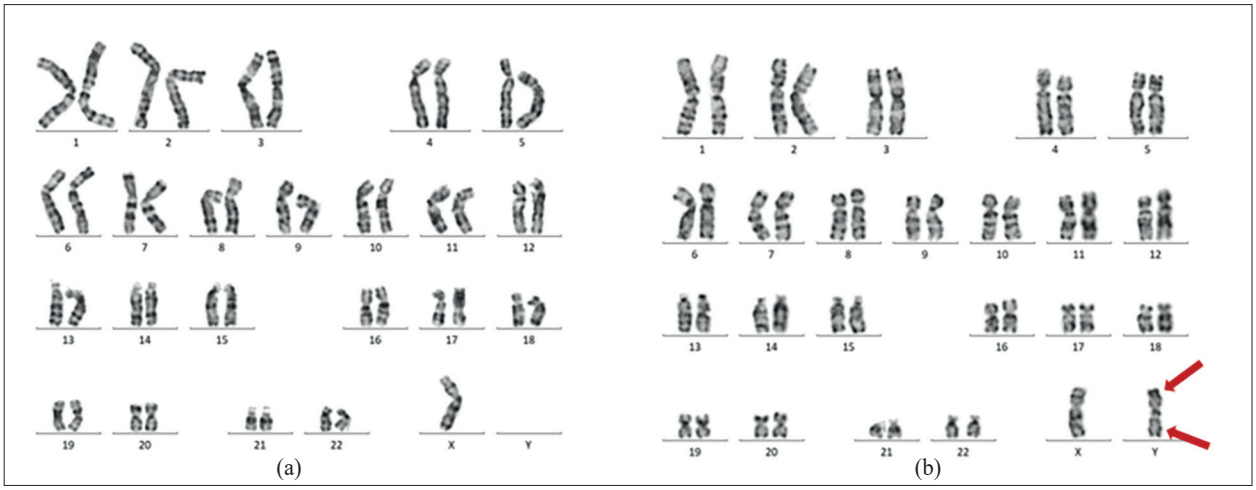

Figure 1: Giemsa banding showed a karyotype of 45,X from peripheral blood lymphocytes (a) and a karyotype of 46,XY from the epithelial cells of the oral mucosa (b).

cliteromegaly. Prader staging was consistent with stage 1 and excessive lanugo pubescence was also observed, which differed from typical Turner syndrome symptoms. The baby underwent surgery for aortic coarctation. Based on the clinical information provided, the marker chromosome observed in the karyotype of the patient is likely the mosaic chromosome Y. The results are consistent with the genetic diagnosis of 45,X/46,XY mixed gonadal dysgenesis Lastly, genetic counselling was offered to the family.

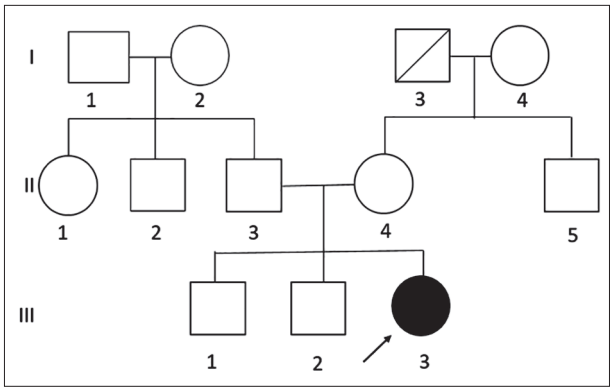

Figure 2: Pedigree of the family in this case study. No numerical and/or structural anomalies were observed in the karyotypes of parents and siblings.

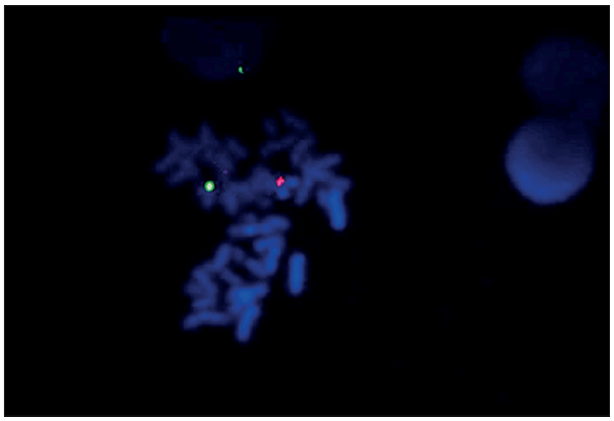

Figure 3: Application of -FISH to confirm the presence of Y chromosome. *SRY* gene duplication on Y chromosome was analyzed by FISH. A positive SRY signal shown by the red light is found at the terminal Y p-arm. The centromere of the X chromosome is shown by the green light.

DISCUSSION

Genome-wide studies allow scientists to detect fetal and maternal (mosaic) aneuploidies [4,13]. According to different studies, sequencing cffDNA from maternal plasma can be helpful to detect all fetal chromosomal aneuploidies, segmental imbalances, and submicroscopic copy number variations (CNVs). However, it has been pointed out that NIPT is not routinely used for screening sex chromosomal aneuploidies (SCAs) because different phenotypes are observed in SCAs, such as monosomy X (Turner syndrome), XXY (Klienefelter syndrome), XYY and XXX, and some of the phenotypes re not detected until adulthood due to fertility problems [4,7,11]. Moreover, the use of NIPT for the detection of mosaicism has not been fully elucidated.

In this particular study, we reported on a case whose NIPT diagnosis was originally 45,X and who was diagnosed with mixed gonadal dysgenesis 45,X/46,XY after birth. A 38-year-old [G3P3] pregnant woman underwent NIPT at 15 weeks' gestation and was found to be at probable risk for 45,X. The pregnant woman did not want to undergo cordocentesis due to it is an invasive procedure. Subsequently, karyotyping of peripheral blood and oral mucosal epithelial cells was performed after birth and the baby's karyotype was determined as 45,X/46,XY+mar? respectively. In addition, *SRY* gene duplication was detected by fluorescence in situ hybridization (FISH) and microarray analysis. Based on the findings from the microarray analysis in the analyzed sample, one copy of the X chromosome was detected in all cells and the presence of one copy of the Y chromosome was detected in a ~40% mosaic state: arr(X)x1,(Y)x1[0.4]. The patient's clinical examination showed ambiguous genitalia (clitoromegaly) and dysmorphic facial features. Based on the clinical information provided, the marker chromosome observed in the karyotype of the patient is likely the mosaic chromosome Y. The results are consistent with the genetic diagnosis of 45,X/46,XY mixed gonadal dysgenesis.

Individuals with mixed gonadal dysgenesis (MGD) exhibit chromosomal mosaicism as well as dysgenetic gonads and different internal and external reproductive morphology [14]. Because of clinical variance, the true prevalence of MGD is unclear. In our case, the ovaries were examined and they were normally formed.

While mixed gonadal dysgenesis (MGD) is cytogenetically 45,X/46,XY, Turner syndrome is generally defined as 45,X. Patients with 45,X/46,XY show a wide range of phenotypes, including phenotypically normal males and Turner syndrome [15,16]. Cardiovascular anomalies such as bicuspid aortic valve, hypertension, aortic coarctation, dilatation of the growing aorta are observed in 20-40% of patients with MGD [17,18]. In a different study, it was stated that patients who have MGD and whose karyotype was characterized by 45,X/46,XY were known to have complications of Turner syndrome. In that study, a 16-year-old social male with MGD was reported to develop coarctation of the aorta, which is one of the most common problems in Turner syndrome [19]. In our study, the baby underwent surgery for aortic coarctation.

The physical appearence of an individual relies on anatomical sex analysis. Karyotype analysis, however, reveals the choromosomal sex which is mainly used to classify sex developmental disorders. In these diseases, although different conditions are detected, 45,X/46,XY mosaicism, known as mixed gonadal dysgenesis, is commonly observed. It has been hypothesized that this mosaicism occurs mainly due to the loss of the Y chromosome because of the non-disjunction that occurs during normal disomic fertilization [20].

Moreover, Mosaic Turner syndrome cases may arise incidentally during divisions that occur in early embryogenesis, at cleavage stage. Patients with mosaic Turner syndrome have a mosaic karyotype of 45,X/46,XX, 46,X,i(Xq) and other variants [1]. Genotype 45,X/46,XY is observed in nearly 10-12% of patients with Turner syndrome [21]. The mosaic Turner Syndrome may be underdiagnosed due to several reasons, such as subtle phenotypic characteristics and technical problems [12]. This is especially common in patients who have low rates of mosaicism due to an increased number of euploid cells that may affect the true genetic diagnosis of Turner Syndrome [1]. Mosaicism, which is unique to the fetus, and the very low amounts of Y chromosomes in maternal blood could explain false-negative results for the presence of a Y chromosome upon NIPT [6,22]. Because the distribution and proportion of euploid and aneuploid cells are different in mosaic pregnancies, these could be important biological factors which reduce the effective DNA proportion, thus obtaining a false-negative aneuploidy [10].

In addition, Hayata et al., (2016) point out that most cffDNA found in maternal blood plasma is derived from trophoblastic cells of the placenta and not from the fetus. Therefore, NIPT cannot provide a definitive diagnosis [23]. In another study, it was discussed that false negative NIPT results occur due to chromosomal mosaicism. It was mentioned that two different mosaicisms are associated with a normal karyotype in the cytotrophoblast, while the fetus itself has a chromosomal abnormality [13]. The first type of mosaicism is generalized mosaicism confined direct normality (GMDD), which is defined by the presence of a chromosomal abnormality in the fetus and the mesechymal origin of the placenta, with the cytotrophoblast being chromosomally not abnormal. On the other hand, the very rare confined fetal mosaicism (CFM) shows a normal karyotype in the villi of short-term and long-term culture and abnormal cytogenetic results in the fetus. Importantly, both types of mosaicism show normal NIPT results because the karyotype in the cytotrophoblast is normal, although the fetus has an abnormal karyotype. Also, it has been pointed out that a normal NIPT result may be a false negative result [13,24].

Importantly, each test and diagnostic procedure has its own benefits and risks. Most prenatal genetic screening tests are designed to reduce the risk of invasive procedures. Analysis of karyotype by amniocentesis remains as the most common method for detecting SCAs and the golden standard for cytogenetic diagnosis [4,9]. In addition, prenatal diagnosis of SCAs in a fetus might be complicated due to several reasons, one of which is the lack of confirmatory ultrasound findings beyond increased nuchal translucency. Additionally, maternal factors such as SCA mosaicism or age-related loss of the X chromosome can affect the interpretation of the data, causing false positive cfNIPT results. Furthermore, it has been emphasized that when the fetus-driven cell-free DNA concentration is low in the blood, non-invasive prenatal genetic testing is not determinate [7].

Moreover, it has been indicated that cytogenetic analysis may not be sufficient to detect Y-chromosome material since it may be found in a small number of cells in small amounts or even as part of marker chromosomes containing Y-specific regions [5,25]. Thus, other techniques such as molecular analysis should be used for the accurate diagnosis of Turner Syndrome. The percentage distribution of mosaicism in different tissues, which differs between blood and gonadal tissue, determines the phenotype of a 45,X/46,XY mosaic patient [7,8].

According to the literature, although NIPT has been introduced to detect sex chromosomal abnormalities, these tests are far from replacing invasive diagnostic procedures. Moreover, in several studies it has been stated that mosaicism cannot be confirmed by NIPT [4,26]. For example, one study reported that NIPT and karyotyping results were inconsistent in Turner syndrome (TS). In summary, a 35-year-old pregnant woman underwent NIPT and showed probable risk for Xp deletion. However, amniocentesis was performed and after cytogenetic analysis the karyotype was determined as mos 45,X [28]/46,X,i(X)(q1.0) [5]. In the second case, a 33-year-old woman underwent NIPT and showed a probable risk of monosomy X. However, amniocentesis was performed and after the cytogenetic analysis the karyotype was determined as mos 45,X [8]/46,XY[8] [4].

Importantly, if the fetus is detected at high risk for aneuploidy, further testing is required to confirm the NIPT result. Positive NIPT results for sex chromosome abnormalities should be carefully evaluated and confirmed with an invasive procedure [4,22]. Therefore, it is crucial that women interested in NIPT should be fully informed about the procedure, its benefits, and limitations. Furthermore, pregnant women who have had a positive ultrasound result are strongly advised to undergo invasive prenatal testing [10,11].

In invasive prenatal diagnostics, the use of chromosome microarray analysis, and more recently next-generation sequencing approaches, has significantly expanded the prenatal diagnostic yield. According to the literature, next-generation sequencing approaches should be used in addition to normal routine testing including karyotype and/or chromosome microarray analysis and not as the first diagnostic test for fetal abnormalities. When using these new techniques, parental counselling should be mandatory before and after testing, and close collaboration in a multidisciplinary team is strongly recommended [4,11].

In conclusion, although NIPT has been routinely used to screen numerical abnormalities including trisomy 21,18 and 13, it still has potential limitations in correctly identifying sex chromosomes and mosaicism that may mislead clinicians and families. In routine clinical practise, conventional cytogenetic analysis following invasive prenatal testing remains as an important component of prenatal care.

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